AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph under "Related Applications" at page 1 of the specification with the following amended paragraph:

This application is a continuation application of U.S. Application Serial No. 09/881,797, filed June 14, 2001, and claims the benefit ~~continuation-in-part~~ of a provisional application of U.S. Application Serial Number 60/211,749, filed June 14, 2000, both of which [[is]]are incorporated by reference in [[its]]their entirety.

Please replace the paragraph at page 2, under "Background" with the following amended paragraph:

Asthma has been linked to markers on human chromosome 12 (Wilson et al., 1998, *Genomics*, **53**: 251-259). In addition, obesity has been linked to asthma (Wilson et al., 1999, *Arch. Intern. Med.* **159**: 2513-14). In particular, chromosomal region 12q23-qter has been associated with a variety of genetic disorders, including male germ cell tumors, histidinemia, growth retardation with deafness and mental retardation, deficiency of Acyl-CoA dehydrogenase, spinal muscular atrophy, Darier disease, cardiomyopathy, Spinocerebellar ataxia-2, brachydactyly, Mevalonicaciduria, Hyperimmunoglobulinemia D, Noonan syndrome-1, Cardiofaciocutaneous syndrome, spinal muscular atrophy-4, tyrosinemia, phenylketonuria, B-cell non-Hodgkin lymphoma, Ulnar-mammary syndrome, Holt-Oram syndrome, Scapuloperoneal spinal muscular atrophy, alcohol intolerance, MODY, Diabetes mellitus, noninsulin-dependent 2, and diabetes mellitus insulin-dependent (See National Center for Biotechnology Information; Bethesda, MD; ~~http://www.ncbi.nlm.nih.gov/omim/~~). The genes of this regions are also associated with obesity, lung disease, particularly, inflammatory lung disease phenotypes such as Chronic Obstructive Lung Disease (COPD), Adult Respiratory Distress Syndrome (ARDS), and asthma. However, few genes in chromosomal region 12q23-qter have been discovered. Thus, there is a need in the art for the identification of specific genes that are involved in these disorders. Identification and characterization of such genes will allow the development of effective diagnostics and therapeutic means to diagnose, prevent, and/or treat lung related disorders, as well as the other diseases described herein.

Please replace the paragraphs under "Brief Description of the Figures" at page 6 of the specification with the following amended paragraphs:

Figures 2A-2P show genes mapped to the 12q23-qter interval determined from information that is curated by the National Center for Biotechnology Information, "NCBI" (~~http://www.ncbi.nlm.nih.gov/genemap/~~Bethesda, MD). This particular information contains genes mapped against the Gene Bridge (GB) 4 panel.

Figures 3A-3G show genes mapped to the 12q23-qter interval determined from information that is curated by NCBI (~~http://www.ncbi.nlm.nih.gov/genemap/~~Bethesda, MD). This particular information contains genes mapped against the Gene Bridge (GB) 3 panel.

Figure 4 shows the integration of the Marshfield Center for Medical Genetics (~~http://www.marshmed.org/genetics/~~Marshfield, MI) genetic map with GeneMap99 from NCBI. The regions of study mentioned above are indicated at the top of the figure.

Please replace the paragraph bridging pages 29 and 30 with the following amended paragraph:

General methods for splice site prediction can be found in Nakata, 1985, *Nucleic Acids Res.* **13**:5327-5340. In addition, splice sites can be predicted using, for example, the GRAIL™ (E.C. Uberbacher and R.J. Mural, 1991, *Proc. Natl. Acad. Sci. USA*, **88**:11261-11265; E.C. Uberbacher, 1995, *Trends Biotech.*, **13**:497-500; ~~http://grail.lsd.ornl.gov/grailexp~~); GenView (L. Milanesi et al., 1993, *Proceedings of the Second International Conference on Bioinformatics, Supercomputing, and Complex Genome Analysis*, H.A. Lim et al. (eds), World Scientific Publishing, Singapore, pp. 573-588; ~~http://125.itba.mi.cnr.it/~webgene/wwwgene_help.html~~); SpliceView (~~http://www.itba.mi.cnr.it/webgene~~ The Institute of Biomedical Technologies I.T.B.; Italy); and HSPL (V.V. Solovyev et al., 1994, *Nucleic Acids Res.* **22**:5156-5163; V.V. Solovyev et al., 1994, "The Prediction of Human Exons by Oligonucleotide Composition and Discriminant Analysis of Spliceable Open Reading Frames," R. Altman et al. (eds), *The Second International conference on Intelligent systems for Molecular Biology*, AAAI Press, Menlo Park, CA, pp. 354-362; V.V. Solovyev et al., 1993, "Identification Of Human Gene Functional Regions Based On

Oligonucleotide Composition," L. Hunter et al. (eds), *In Proceedings of First International conference on Intelligent System for Molecular Biology*, Bethesda, pp. 371-379) computer systems.

Please replace the paragraph at page 30 with the following amended paragraph:

Additionally, computer programs such as GeneParser (E.E. Snyder and G.D. Stormo, 1995, *J. Mol. Biol.* **248**: 1-18; E.E. Snyder and G.D. Stormo, 1993, *Nucl. Acids Res.* **21**(3): 607-613~~; http://mcdb.colorado.edu/~eesnyder/ GeneParser.html~~Boulder, CO); MZEF (M.Q. Zhang, 1997, *Proc. Natl. Acad. Sci. USA*, **94**:565-568~~; http://argon.cshl.org/genefinder~~Cold Spring Harbor Laboratory; Cold Spring Harbor, NY); MORGAN (S. Salzberg et al., 1998, *J. Comp. Biol.* **5**:667-680; S. Salzberg et al. (eds), 1998, *Computational Methods in Molecular Biology*, Elsevier Science, New York, NY, pp. 187-203); VEIL (J. Henderson et al., 1997, *J. Comp. Biol.* **4**:127-141); GeneScan (S. Tiwari et al., 1997, *CABIOS (BioInformatics)* **13**: 263-270); GeneBuilder (L. Milanesi et al., 1999, *Bioinformatics* **15**:612-621); Eukaryotic GeneMark (J. Besemer et al., 1999, *Nucl. Acids Res.* **27**:3911-3920); and FEXH (V.V. Solovyev et al., 1994, *Nucleic Acids Res.* **22**:5156-5163). In addition, splice sites (i.e., former or potential splice sites) in cDNA sequences can be predicted using, for example, the RNASPL (V.V. Solovyev et al., 1994, *Nucleic Acids Res.* **22**:5156-5163); or INTRON (A. Globek et al., 1991, INTRON version 1.1 manual, Laboratory of Biochemical Genetics, NIMH, Washington, D.C.) programs.

Please replace the paragraph at page 43, lines 4-13, with the following amended paragraph:

As non-limiting examples, antisense oligonucleotides may be targeted to hybridize to the following regions: mRNA cap region; translation initiation site; translational termination site; transcription initiation site; transcription termination site; polyadenylation signal; 3' untranslated region; 5' untranslated region; 5' coding region; mid coding region; and 3' coding region. Preferably, the complementary oligonucleotide is designed to hybridize to the most unique 5' sequence of a 12q23-qter gene, including any of about 15-35 nucleotides spanning the 5' coding sequence. Appropriate oligonucleotides can be designed using OLIGO software (Molecular Biology Insights, Inc., Cascade, CO~~; http://www.oligo.net~~).

Please replace the first paragraph under "Structural Studies," which bridges pages 51 and 52, with the following amended paragraph:

A purified 12q23-qter polypeptide (e.g., SEQ ID NO:93 to SEQ ID NO:155), or portions or complexes thereof, can be analyzed by well-established methods (e.g., X-ray crystallography, NMR, CD, etc.) to determine the three-dimensional structure of the molecule. The three-dimensional structure, in turn, can be used to model intermolecular interactions. Exemplary methods for crystallization and X-ray crystallography are found in P.G. Jones, 1981, *Chemistry in Britain*, **17**:222-225; C. Jones et al. (eds), *Crystallographic Methods and Protocols*, Humana Press, Totowa, NJ; A. McPherson, 1982, *Preparation and Analysis of Protein Crystals*, John Wiley & Sons, New York, NY; T.L. Blundell and L.N. Johnson, 1976, *Protein Crystallography*, Academic Press, Inc., New York, NY; A. Holden and P. Singer, 1960, *Crystals and Crystal Growing*, Anchor Books-Doubleday, New York, NY; R.A. Laudise, 1970, *The Growth of Single Crystals*, Solid State Physical Electronics Series, N. Holonyak, Jr., (ed), Prentice-Hall, Inc.; G.H. Stout and L.H. Jensen, 1989, *X-ray Structure Determination: A Practical Guide*, 2nd edition, John Wiliey & Sons, New York, NY; *Fundamentals of Analytical Chemistry*, 3rd. edition, Saunders Golden Sunburst Series, Holt, Rinehart and Winston, Philadelphia, PA, 1976; P.D. Boyle of the Department of Chemistry of North Carolina State University ~~at http://laue.chem.ncsu.edu/web/GrowXtal.html~~; M.B. Berry, 1995, *Protein Crystalization: Theory and Practice, Structure and Dynamics of E. coli Adenylate Kinase*, Doctoral Thesis, Rice University, Houston TX~~; www.bioc.rice.edu/~berry/papers/crystalization/crystalization.html~~.

Please replace the paragraph bridging pages 54 and 55 with the following amended paragraph:

A crystallization protocol can be adapted to a particular polypeptide or peptide. In particular, the physical and chemical properties of the polypeptide can be considered (e.g., aggregation, stability, adherence to membranes or tubing, internal disulfide linkages, surface cysteines, chelating ions, etc.). For initial experiments, the standard set of crystalization reagents can be used (Hampton Research, Laguna Niguel, CA). In addition, the CRYSTOOL program

can provide guidance in determining optimal crystallization conditions (Brent Segelke, 1995, Efficiency analysis of sampling protocols used in protein crystallization screening and crystal structure from two novel crystal forms of PLA2, Ph.D. Thesis, University of California, San Diego; ~~http://www.ccp14.ac.uk/ccp/web-mirrors/llnlrupp/crystool/crystool.htm~~). Exemplary crystallization conditions are shown below (see Berry, 1995).

Please replace the paragraph at page 73 under "Diagnostics" with the following amended paragraph:

As discussed herein, 12q23-qter genes are associated with various diseases and disorders, including but not limited to, asthma, atopy, obesity, male germ cell tumors, histidinemia, growth retardation with deafness and mental retardation, deficiency of Acyl-CoA dehydrogenase, spinal muscular atrophy, Darier disease, cardiomyopathy, Spinocerebellar ataxia-2, brachydactyly, Mevalonicaciduria, Hyperimmunoglobulinemia D, Noonan syndrome-1, Cardiofaciocutaneous syndrome, spinal muscular atrophy-4, tyrosinemia, phenylketonuria, B-cell non-Hodgkin lymphoma, Ulnar-mammary syndrome, Holt-Oram syndrome, Scapuloperoneal spinal muscular atrophy, alcohol intolerance, MODY, diabetes mellitus, non-insulin-dependent type 2, diabetes mellitus insulin-dependent (See National Center for Biotechnology Information; Bethesda, MD ~~at http://www.ncbi.nlm.nih.gov/omim/~~), and inflammatory bowel disease (B. Wallaert et al., 1995, *J. Exp. Med.* **182**:1897-1904). The present invention therefore provides nucleic acids and antibodies that can be useful in diagnosing individuals with disorders associated with aberrant 12q23-qter gene expression and/or mutated 12q23-qter genes. In particular, nucleic acids comprising 12q23-qter SNPs can be used to identify chromosomal abnormalities linked to these diseases. Additionally, antibodies directed against the amino acid variants encoded by the 12q23-qter SNPs can be used to identify disease-associated polypeptides.

Please replace the paragraph at page 87 under "Therapeutics" with the following amended paragraph:

As discussed herein, 12q23-qter genes are associated with various diseases and disorders, including but not limited to, asthma, atopy, obesity, male germ cell tumors, histidinemia, growth retardation with deafness and mental retardation, deficiency of Acyl-CoA

dehydrogenase, spinal muscular atrophy, Darier disease, cardiomyopathy, Spinocerebellar ataxia-2, brachydactyly, Mevalonicaciduria, Hyperimmunoglobulinemia D, Noonan syndrome-1, Cardiofaciocutaneous syndrome, spinal muscular atrophy-4, tyrosinemia, phenylketonuria, B-cell non-Hodgkin lymphoma, Ulnar-mammary syndrome, Holt-Oram syndrome, Scapuloperoneal spinal muscular atrophy, alcohol intolerance, MODY, diabetes mellitus, non-insulin-dependent type 2, diabetes mellitus insulin-dependent (See National Center for Biotechnology Information ~~at http://www.ncbi.nlm.nih.gov/omim/~~), and inflammatory bowel disease (B. Wallaert et al., 1995, *J. Exp. Med.* **182**:1897-1904). The present invention therefore provides compositions (e.g., pharmaceutical compositions) comprising 12q23-qter nucleic acids, polypeptides, antibodies, ligands, or variants, portions, or fragments thereof that can be useful in treating individuals with these disorders. Also provided are methods employing 12q23-qter nucleic acids, polypeptides, antibodies, ligands, or variants, portions, or fragments thereof to identify drug candidates that can be used to prevent, treat, or ameliorate such disorders.

Please replace the paragraph at page 117, lines 18-28 with the following amended paragraph:

An additional 63 families from the United Kingdom were utilized from an earlier collection effort with different ascertainment criteria. These families were recruited either: 1) without reference to asthma and atopy; or 2) by having at least one family member or at least two family members affected with asthma. The randomly ascertained samples were identified from general practitioner registers in the Southampton area. For families with affected members, the probands were recruited from hospital based clinics in Southampton. Seven pedigrees extended beyond a single nuclear family. The phenotypic and genotypic data information for 17 markers for 21 of these 63 families was obtained from the website http://cedar.genetics. soton.ac.uk/pub /PROGRAMS/BETA/data/bet12.ped.

Please replace the paragraph at page 121, lines 9-23 with the following amended paragraph:

1. Asthma Phenotype: For the initial linkage analysis, the phenotype and asthma affection status were defined by a patient who answered the following questions in the affirmative: i) Have you ever had asthma? ii) Do you have a current physician's diagnosis of

asthma? and iii) Are you currently taking asthma medications? Medications included inhaled or oral bronchodilators, cromolyn, theophylline, or steroids. Multipoint linkage analyses of allele sharing in affected individuals were performed using the MAPMAKER/SIBS analysis program (L. Kruglyak and E.S. Lander, 1995, *Am. J. Hum. Genet.* 57:439-454). The analyses were performed using 54 polymorphic markers spanning a 162 cM region on both arms of chromosome 12. The map location and distances between markers were obtained from the genetic maps published by the Marshfield medical research foundation (~~http://www.marshmed.org/genetics/~~Marshfield, MI). Ambiguous ordering of markers in the Marshfield map was resolved using the program MULTIMAP (T.C. Matise et al., 1994, *Nature Genet.* 6:384-390).

Please replace the paragraph at page 125, lines 19-25 with the following amended paragraph:

Physical mapping (BAC contig construction) focused on a ~22 cM interval approximately between markers D12S307 and D12S2341. The discovery of novel genes using direct cDNA selection focused on a ~15 cM region between markers D12S1609 and D12S357. Figure 4 shows the integration of the Marshfield Center for Medical Genetics (~~http://www.marshmed.org/genetics/~~) genetic map with GeneMap99 from NCBI. The relevant regions are indicated at the top of the figure.

Please replace the paragraphs at page 126, line 5 through page 127, line 3, with the following amended paragraphs:

Figures 5A-5I show the BAC/STS content contig map of human chromosome 12q23-qter. Markers used to screen the RPCI-11 BAC library (P. deJong, Roswell Park Cancer Institute (RPCI)) are shown in the top row. Markers that were present in the Genome Database (GDB, Research Triangle Institute (RTI) International; Research Triangle, NC~~http://gdbwww.gdb.org/~~) are represented by GDB nomenclature. The BAC clones are shown below the markers as horizontal lines.

1. Map Integration. Various publicly available mapping resources were utilized to identify existing STS (sequence tagged site) markers in the 12q23-qter region (Olson

et al., 1989, *Science*, **245**:1434-1435). Resources included GDB ~~(http://gdbwww.gdb.org/)~~, Genethon (France~~http://www.genethon.fr/genethon_en.html~~), the Marshfield Center for Medical Genetics ~~(http://www.marshmed.org/genetics/)~~, the Whitehead Institute Genome Center (Cambridge, MA~~http://www.genome.wi.mit.edu/~~), GeneMap98, dbSTS, and dbEST (NCBI, ~~http://www.ncbi.nlm.nih.gov/~~), the Sanger Centre (United Kingdom~~http://www.sanger.ac.uk/~~), and the Stanford Human Genome Center (Stanford, CA~~http://www.shgc.stanford.edu/~~). Maps were integrated manually to identify markers mapping to the disorder region. A list of markers is shown in Table 2.

2. Marker Development: Sequences for existing STSs were obtained from the GDB, Radiation Hybrid Database~~RHDB~~ (RHDB; United Kingdom ~~http://www.ebi.ac.uk/RHdb/~~), or NCBI, and were used to pick primer pairs (overgos; see Table 2) for BAC library screening. Novel markers were developed from publicly available genomic sequences, proprietary cDNA sequences, or from sequences derived from BAC insert ends (described below). Primers were chosen using a script that automatically performs vector and repetitive sequence masking using CROSSMATCH (P. Green, University of Washington). Subsequent primer selection was performed using a customized Filemaker Pro database (Filemaker, Inc.; Santa Clara, CA~~http://www.filemaker.com~~). Primers for use in PCR-based clone confirmation or radiation hybrid mapping (described below) were chosen using the program Primer3 (Steve Rozen, Helen J. Skaletsky, 1996, 1997, Rozen, S., Skaletsky, H. "Primer3 on the WWW for general users and for biologist programmers." In S. Krawetz and S. Misener, eds. Bioinformatics Methods and Protocols in the series Methods in Molecular Biology. Humana Press, Totowa, NJ, 2000, pages 365-386. ~~http://www-genome.wi.mit.edu/genome_software/other/primer3.html~~).

Please replace the paragraphs at page 138, line 15 through page 139, line 5, with the following amended paragraphs:

Images of the gels were captured with a Kodak DC40 CCD camera and processed with Kodak 1D software (Eastman Kodak Comp.; Rochester, NY~~www.kodak.com~~). The gel data were exported as tab delimited text files. The names of the files included information about the panel screened, the gel image files, and the marker screened. These data were automatically imported using a customized Perl script into Filemaker databases for data storage and analysis.

The data were then automatically formatted and submitted to an internal server for linkage analysis to create a radiation hybrid map using RHMAPPER (L. Stein et al., 1995; available from Whitehead Institute/MIT Center for Genome Research, ~~at http://www.genome.wi.mit.edu/ftp/pub/software/ rhmapper/, and via anonymous ftp to ftp.genome. wi.mit.edu, in the directory /pub/software/rhmapper.~~)

4. BAC Library Screening: The protocol used for BAC library screening was based on the "overgo" method, originally developed by John McPherson at Washington University in St. Louis (http://www.tree.caltech .edu/protocols/overgo.html, and W-W. Cai et al., 1998, *Genomics* **54**:387-397). This method involved filling in the overhangs generated after annealing two primers. Each primer was 22 nucleotides in length, and overlapped by 8 nucleotides. The resulting labeled product (36 bp) was then used in hybridization-based screening of high density grids derived from the RPCI-11 BAC library (deJong, *supra*). Typically, 15 probes were pooled together to hybridize 12 filters (13.5 genome equivalents).

Please replace the bridging paragraph at page 141, line 27 through page 142, line 10, with the following amended paragraphs:

The supernatant was transferred carefully (avoiding the white precipitate) to a fresh 2 ml Eppendorf tube, and 0.9 ml of isopropanol was added. The solution was mixed and left on ice for 5 min. The samples were centrifuged for 10 min, and the supernatant was carefully removed. Pellets were washed in 70% ethanol and air-dried for 5 min. Pellets were then resuspended in 200 µl of TE8 (10 mM Tris-HCl, pH 8.0, 1.0 mM EDTA, pH 8.0), and RNase (Boehringer Mannheim; Germany, ~~http://biochem.boehringer-mannheim.com~~) added to 100 µg/ml. Samples were incubated at 37°C for 30 min, then precipitated by addition of NH_4OAc to 0.5 M and 2 volumes of ethanol. Samples were then centrifuged for 10 min, and the pellets were washed with 70% ethanol. The pellets were air-dried and dissolved in 50 µl TE8. Typical yields for this DNA prep were 3-5 µg per 15 ml bacterial culture. Ten to 15 µl of DNA was used for *EcoRI* restriction analysis; 5 µl was used for *Not*I digestion and clone insert sizing by CHEF gel electrophoresis.

Please replace the paragraph at page 149, lines 4-11 with the following amended paragraph:

b. BAC vector sequences were masked within the sequence by using the program CROSSMATCH (P. Green, University of Washington; Seattle, WA~~http:\\chimera.biotech. washington.edu\UWGC~~). Shotgun library construction (detailed above) left BAC vector sequences in the shotgun libraries. The CROSSMATCH program was used to compare the sequence of the BAC contigs to the BAC vector and to mask any vector sequence prior to subsequent steps. Masked sequences were marked by "Xs" in the sequence files, and were omitted during subsequent analyses.

Please replace the paragraph at page 153, lines 3-24, with the following amended paragraphs:

1. Construction and screening of cDNA libraries: Directionally cloned cDNA libraries from normal lung and bronchial epithelium were constructed using standard methods (Soares et al., 1994, *Automated DNA Sequencing and Analysis*, Adams et al. (eds), Academic Press, NY, pp. 110-114). Total and cytoplasmic RNAs were extracted from tissue or cells by homogenizing the sample in the presence of guanidinium thiocyanate-phenol-chloroform extraction buffer (e.g., Chomczynski and Sacchi, 1987, *Anal. Biochem.* **162**:156-159) using a polytron homogenizer (Brinkman Instruments, Inc.; Westbury, NY~~; http://www.brinkmann.com~~). Poly (A)+ RNA was isolated from total/cytoplasmic RNA using dynabeads-dT according to the manufacturer's recommendations (Dynal Biotech; Norway~~, Inc., http://www.dynal.com~~). The double stranded cDNA was then ligated into the plasmid vector pBluescript II KS+ (Stratagene, La Jolla, CA~~http://www.stratagene.com~~), and the ligation mixture was transformed into *E. coli* host DH10B or DH12S by electroporation (Soares, 1994). Following overnight growth at 37°C, DNA was recovered from the *E. coli* colonies after scraping the plates as directed for the Mega-prep kit (QIAGEN). The quality of the cDNA libraries was estimated by counting a portion of the total number of primary transformants, determining the average insert size, and calculating the percentage of plasmids without cDNA insert. Additional cDNA libraries (human total brain, heart, kidney, leukocyte, and fetal brain) were purchased from Life Technologies (Bethesda, MD).

Please replace the paragraph at page 192, lines 1-15 with the following amended paragraph:

1. RFLP Assay: The amplicon containing the polymorphism was PCR amplified using primers that generated fragments for sequencing (sequencing primers) or SSCP (SSCP primers). The appropriate population of individuals was PCR amplified in 96-well microtiter plates. Enzymes were purchased from NEB. The restriction cocktail containing the appropriate enzyme for the particular polymorphism was added to the PCR product. The reaction was incubated at the appropriate temperature according to the manufacturer's recommendations for 2-3 hr, followed by a 4°C incubation. After digestion, the reactions were size fractionated using the appropriate agarose gel depending on the assay specifications (2.5%, 3%, or Metaphor, FMC Bioproducts). Gels were electrophoresed in 1 X TBE buffer at 170 V for approximately 2 hr. The gel was illuminated using UV, and the image was saved as a Kodak 1D file. Using the Kodak 1D image analysis software, the images were scored and the data was exported to Microsoft® Excel (Microsoft Corp.; Redmond, WA~~http://www.microsoft.com~~).

Please replace the bridging paragraph at page 193, line 26 to page 194, line 8, with the following amended paragraph:

The ASO was removed from the filter by adding 1 L of boiling strip solution (0.1 x SSPE (pH 7.4) and 0.1% SDS). This was repeated two more times. After removing the ASO, the filter was pre-hybridized in 300 ml pre-hybridization solution (5 X SSPE (pH 7.4), 2% SDS, and 5 X Denhardt's) at 40°C for over 1 hr. The second end-labeled ASO corresponding to the other strand was removed from storage at -20°C and thawed at RT. The filter was placed into a glass bottle along with 10 ml hybridization solution and the entire end-labeled product of the second ASO. The hybridization reaction was placed in a rotisserie oven (Hybaid Limited, United Kingdom~~http://www.hybaid.co.uk~~) and left at 40°C for a minimum of 4 hr. After the hybridization, the filter was washed at various temperatures and images captured on film as described above. The best image for each ASO was converted into a digital image by scanning the film into Adobe® Photoshop®. These images were overlaid using Graphic Converter, and the overlaid images were scored.